Exposure of Wolves to Canine Parvovirus and Distemper on the Kenai National Wildlife Refuge, Kenai Peninsula, Alaska, 1976–1988

■ Theodore N. Bailey, Edward E. Bangs, and Rolf O. Peterson

We tested 55 serum samples from 50 wolves and four from coyotes, live-captured on the Kenai National Wildlife Refuge, Kenai Peninsula, Alaska, between 1976 and 1988, for exposure to canine parvovirus (CPV) and canine distemper virus (CDV). Exposure to CPV was first detected in wolves in December 1979 and increased to a high of 67% of wolves sampled between 1983 and 1984. Exposure to CDV was first detected in wolves in June 1979 and varied from 0% to 67% throughout the testing period. Twelve percent of the sampled wolves had been exposed to both viruses. More males than females had been exposed to CPV; more adults/yearlings than pups had been exposed to CDV. Wolves that had been exposed to both CPV and CDV had signficantly lower hemoglobin levels. The effects of CPV on wolf pup survival were unknown. Only one of four tested coyotes had been exposed to CPV and none to CDV. Numerous domestic dogs adjacent to the refuge are a continual source of both diseases.

Introduction

Canine parvovirus (CPV) was first recognized and became an important disease among domestic dogs (*Canis familiaris*) in 1978 (Parrish et al. 1985); there was a worldwide epizootic in 1981 (Yang 1987). Serum antibodies to CPV were first reported in wolves (*C. lupus*) in Alaska in 1980 (Zarnke and Ballard 1987). Prevalence of CPV exposure in wolves from the Nelchina Basin in mainland Alaska was 31% (n = 32) between 1975 and 1982, but no mortality among adult wolves was attributed to the disease (Zarnke and Ballard 1987).

The first wolf mortality attributed to canine distemper virus (CDV) in Alaska was a yearling male from the Kenai National Wildlife Refuge (KNWR) found dead on 13 September 1978 (Peterson et al. 1984). An adult female wolf from the same pack was found dead on 15 January 1980 and also diagnosed with CDV. Exposure to CDV among 57 wolves tested throughout mainland Alaska was 7% (Stephenson et al. 1982) and 12% among 12 wolves tested from the Nelchina Basin in mainland Alaska between 1975 and 1982 (Zarnke and Ballard 1987).

Because there was no information on the prevalence of CPV and CDV exposure among wolves and coyotes (*C. l. latrans*) on the Kenai Peninsula, which is almost detached from mainland Alaska, we tested blood samples taken from 50 wolves and four coyotes captured between 1976 and 1988.

Study Area

Wolves and coyotes were live-captured on the KNWR, an area of 7,970 km^2 on the western Kenai Peninsula (KP) in south-central Alaska (Fig. 1). Topography, climate, and vegetation on the KNWR have been previously described (Peterson et al. 1984). Most (85%) serum samples from wolves and all sera from coyotes were collected on the KNWR north of the Kenai River. Eight serum samples were from wolves from the central portion of the KNWR south of the Kenai River and north of the Kasilof River. Most wolf packs, with some exceptions during the latter part of the study period, used territories previously named and described by Peterson et al. (1984); pack names, in parenthesis, are used here for reference.

Size of wolf packs averaged 15.3 during 1976–1977, but declined to 8.0–8.6 by 1981–82 due to increasing human harvest (Peterson et al. 1984). Population density ranged from 11.4–19.5 wolves/1,000 km^2. In the early 1980's,

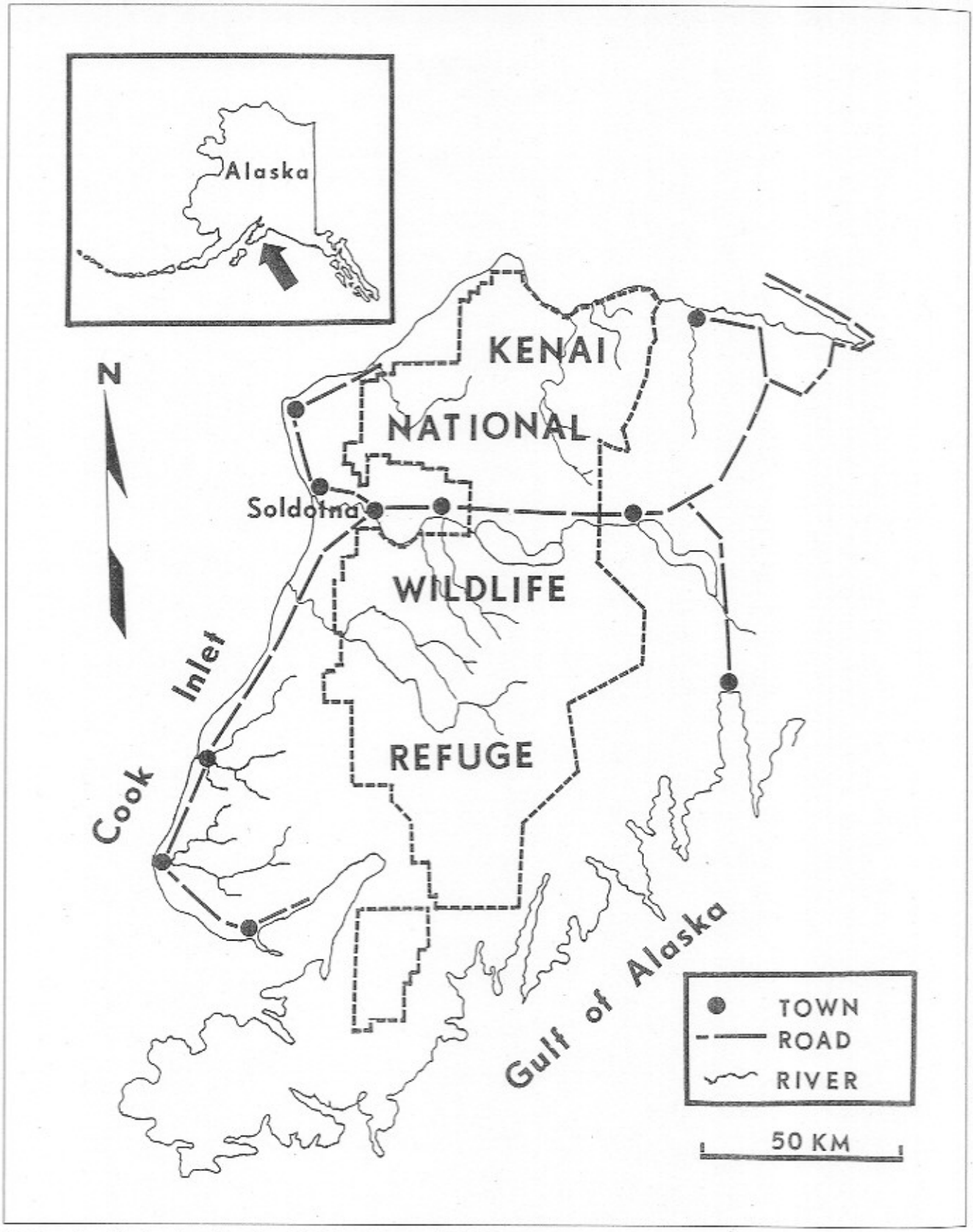

Fig. 1. *Kenai Peninsula and location of the Kenai National Wildlife Refuge. Upper left inset shows location of Kenai Peninsula in Alaska.*

wolves on the KNWR became infested with biting dog lice (*Trichodectes canis*) (Schwartz et al. 1983).

Methods

Wolves were live-trapped from May through October with leg-hold traps, and captured from November through April by means of tranquilizer darts fired from helicopters (Peterson et al. 1984). Coyotes were incidentally captured while trapping for wolves. Both species were weighed and a 1–3 cc sample of whole blood was taken. Packed-cell volume (PCV) values were obtained by centrifuging heparinized whole blood samples in capillary tubes 75 mm long by 0.56 mm in a Clay Adams Readacrit centrifuge. Hemoglobin values were recorded with a Clay Adams Hemoglobinometer.

Fifty-five blood serum samples from 50 individual wolves were tested for exposure to CPV and CDV from 1976 to 1988. Tested wolves were associated with at least nine recognized packs that used the KNWR in 1981 (Peterson et al. 1984). Five wolves were tested twice at approximately seven-, eight-, 12-, 15- and 36-month intervals and four coyotes once between 1976 and 1988.

Sera were aspirated from centrifuged whole blood samples and frozen. Serological tests for evidence of antibodies to CPV using the technique described by Carmichael et al. (1980) were performed at the National Wildlife Health Research Center, Madison, Wisconsin. Sera with titers greater than or equal to 1:320 were considered to be positive evidence of exposure to CPV (or a virus which cross reacts with it).

To test for CDV, sera were diluted 1:5 in Hank's balanced salt solution (BSS) and heat inactivated at 56°C for 30 minutes. Serial twofold dilutions of each serum (1:5 to 1:640) were prepared using Hank's BSS in 96 well plates at 0.05 ml/well. A 0.05 ml solution of reference CDV diluted in Hank's BSS containing 50 infectious units/0.05 ml was added to each serum dilution and incubated 30 minutes at 37°C. African green monkey (*Cercopithecus aethiops*) kidney cells (0.15 ml) were added to each well and the well incubated at 37°C in 2% carbon dioxide for a total of seven days. Test wells were read for vial cytopathic effect every other day for seven days. The titer of each serum was expressed as the last dilution that completely neutralizes the cytopathic effect of CDV. Any titers greater than or equal to 1:5 were considered to be evidence of exposure to CDV. The tests included the following controls: 1) a tissue culture infectious dosage ($TCID_{50}$) of the reference virus stock; 2) a serum control for each serum tested; 3) a cell culture control; and 4) a positive and negative serum control. Sex, age, weight, and blood condition parameters (hemoglobin and packed-cell-volume) from unexposed and exposed wolves were tested for statistical significance ($p<0.050$) using Yates corrected-for-continuity, 2X2 contingency table G-tests and t-tests, respectively (Zar 1984).

Results

Exposure to CPV was not detected among seven wolves that were tested from five separate packs (one pack sampled twice) between 1976 and 1978, or among 12 wolves that were tested from six packs in 1979 (Table 1). The first detection was in an adult female captured 12 December 1979; another adult female and a male pup captured the same day in the same pack (Killey River Pack) tested negative. By 1987, CPV exposure was detected in sera of wolves in six of nine known packs. However, only one wolf per pack was sampled from two of the three packs that tested negative. Wolves from the Skilak Lake Pack were the only wolves to consistently test negative to CPV exposure between 1976 and 1988. With the exception of wolves from the Killey River Pack, once CPV exposure was detected in a wolf in a pack, wolves captured subsequently from the same pack usually tested positive for CPV exposure.

Once detected, exposure of wolves to CPV rapidly increased from 22% during the early period of the study (1979 to 1981) to 67% during the later period (1986 to 1988) (Table 1). Forty-four percent of all wolves sampled between 1979 and 1988 ($n = 48$) tested positive to CPV exposure. Only one of the four coyotes tested had been exposed to CPV, an adult male captured 1 October 1980 near the Swanson River Oilfield. It had a high CPV titer (1:1,280), but appeared to be in good physical condition. Exposure of wolves to CDV between 1976 and 1988 was first detected in an adult female captured 6 June 1979 (Big Indian Creek Pack). No wolves tested prior to 1979 were positive, but in 1979, six of 15 wolves (40%) tested positive (Table 1). Annual prevalence of exposure to CDV among wolves varied considerably between 1976 and 1988. In 1980, three of six wolves tested positive, but in 1984, all tested wolves ($n = 11$) were negative. In 1988, only two wolves were tested but from the same pack (Point Possession Pack); both tested negative. Exposure to CDV was detected among wolves from three of seven wolf packs tested in 1979, but in only one of three packs tested in 1980. All three lone wolves tested between 1976 and 1988 tested positive to CDV exposure (one also tested positive to CPV exposure). All four coyotes tested negative to CDV exposure.

Sex and age differences were detected among wolves testing positive or negative for exposure to CPV and CDV (Table 2). Exposure of wolves to CPV was significantly ($P<0.050$) related to sex; a greater percentage of males (42%) than females (34%) tested positive. Exposure of wolves to CDV was significantly ($P<0.050$) related to age; more adults and yearlings (65%) tested positive than pups (5%).

There was no significant differences in mean PCV values or body weight among wolves that tested positive and negative to exposure to CPV and CDV. However, significantly ($P<0.050$) higher hemoglobin values were found among wolves that tested negative to exposure to

Table 1. Wolf sera and wolf packs tested for canine parvovirus (CPV) and canine distemper virus (CDV) (positive tests/total tested), Kenai National WildLife Refuge, Alaska, 1976-1988.

	WOLF SERA			*WOLF PACKS*	
YEAR	*CPV*	*CDV*	*CPV & CDV*	*CPV*	*CDV*
1976	0/1	0/1	0/1	0/1	0/1
1977	0/4	0/4	0/4	0/3	0/3
1978	0/2	0/2	0/2	0/2	0/2
1979	1/15	6/15	0/15	1/7	4/7
1980	1/6	2/6	1/6	2/3	2/3
1981	1/2	0/2	1/2	1/1	1/1
1983	0/1	0/1	0/1	0/1	0/1
1984	8/11	0/11	0/11	3/4	0/4
1986	1/5	1/5	1/5	2/3	1/3
1987	1/6	1/6	3/6	3/4	3/4
1988	2/2	0/2	0/2	1/1	0/1
Total	**15/55**	**10/55**	**6/55**	-	-

both viruses compared to wolves that tested positive to exposure to both viruses.

Four wolves were retested at seven- to 36-month intervals. Only one wolf, an adult female (Killey River Pack), tested negative for exposure to both viruses each time. Another adult female wolf from the same pack that tested negative to CPV and CDV exposure 11 December 1979 tested positive to exposure to both viruses 2 March 1981. A yearling female (Bear Lake Pack) and a yearling male (Elephant Lake Pack) that had been exposed to CPV when first captured also tested positive to CPV eight and 36 months later, respectively. The yearling male also tested positive to CDV exposure when retested.

An adult male wolf (Elephant Lake Pack) captured 22 June 1986 had the highest measured CPV titer (1:1,280), the lowest recorded PCV value (25.4), and hemoglobin value (11.2). He was blind in the right eye and his pelage had changed from the typical brownish-gray color to almost white compared to his condition on 8 February 1983. At that time, the CPV and hemoglobin values were higher (37 and 17, respectively). He was eventually shot by a hunter at relatively close range near a moose carcass 29 April 1987 despite the fact that most wolves on the KNWR generally avoid humans and are difficult to approach on the ground.

Discussion and Management Implications

We suspect that CPV and a parasite (biting dog lice) were transmitted by domestic dogs into the wolf and coyote population on the KNWR. There was an outbreak of CPV in 1980 among domestic dogs in and near the town of Soldotna, less than 1 km from the KNWR boundary (B. Richards, Richard's Veterinary Clinic, Soldotna, pers. commun.). This followed a similar CPV outbreak among dogs in the Anchorage area. Free-roaming dogs are abundant along the western KNWR boundary. Trappers on and off the refuge report periodically capturing dogs in snares or traps set for wolves and coyotes. Some trappers use dog teams on the refuge to run their traplines,. Between 1989 and 1991, about 500–600 dogs were impounded and approximately 300 were destroyed annually in Soldotna (D. Baxter, Animal Control Officer, Soldotna, pers. commun.). Some years during the early- to mid-1980's, up to 1,000 dogs were impounded annually in Soldotna. We received periodic reports from the public during this period of lone wolves observed near domestic dogs. Radio-collared lone wolves have also been located in or near developed areas inhabited by dogs adjacent to the KNWR. Once CPV or CDV was established among wolf packs, dispersing wolves could have spread both viruses to other packs. All three lone wolves tested had been exposed to CDV and one had been exposed to CPV. The CPV virus has been known to persist in dog feces at room

Table 2. Sexes and ages of wolves testing positive for exposure to canine parvovirus (CPV) and canine distemper virus (CDV) (positive tests/total tested) on the Kenai National WildLife Refuge, Alaska, 1976-1988.

		Serological test results — Exposed to:		
SEX	*AGE*	*CPV*	*CDV*	*CPV & CDV*
Male	adult	2/9	0/9	0/9
	yearling	1/5	1/5	3/5
	pup	5/11	1/11	0/11
	unknown	0/1	0/1	0/1
Female	adult	1/11	4/11	2/11
	yearling	1/8	4/8	1/8
	pup	5/7	0/7	0/7
	unknown	0/3	0/3	0/3
Total		**15/55**	**10/55**	**6/55**

temperature for at least six months and perhaps longer (Pollock 1982, Komolafe 1985).

As did the wolves we sampled, male dogs also appear to have a higher prevalence of exposure to CPV than females (Zupancik et al. 1987). Continual or periodic exposure to CPV was documented among individual wolves for periods of five to 38 months. Because the virus may persist in scats of dogs for up to six months at room temperature (Pollack 1982, Komolafe 1985), investigation by scent-marking wolves of wolf scats containing the virus may periodically expose them to the CPV.

Adult wolves on the KNWR appeared more susceptible to CDV exposure than juveniles. Two wolves, sequentially tested, developed antibodies to CDV after they were at least one year old. Blood serum from an adult wolf later found dead on 15 January 1980 and diagnosed with CDV (Peterson et al. 1984), tested negative for the antibody when captured 68 days earlier.

The effects of CPV and CDV on wolf pup survival on the KNWR was unknown because young pups were not tested. Morbidity and mortality from CPV in dogs were highest in three- to four-month-old pups (Hirasawa et al. 1987). Because the same pattern might be expected to occur among wolf pups (Carbyn 1982b), close monitoring of pups would be necessary to document direct CPV-induced mortality. All wolves tested in this study were at least six months old when tested, and thus were survivors of any early litter mortality.

Carbyn (1982b) believed that diseases, particularly CDV, may have played an important role in reducing the population density of wolves in Riding Mountain National Park, Manitoba, Canada. The KNWR and Riding Mountain National Park wolf populations have some common characteristics. Both are located in areas that are relatively small, isolated from other wolf habitat, and have boundaries where contact between wolves and dogs is common. Immigration and emigration are limited, and human harvest has been a major cause of mortality.

The prevalence of CPV exposure among wolves tested on the KNWR was greater than that previously reported among wolves elsewhere in Alaska (Zarnke and Ballard 1987) or in Minnesota (Mech et al. 1986). Prevalence of CDV exposure was also higher among wolves on the KNWR compared to wolves tested elsewhere in Alaska. Exposure of a wolf to CPV was first detected on the KNWR slightly earlier than reported for wolves elsewhere in Alaska (Zarnke and Ballard 1987).

Immigration of wolves cannot be relied upon to help balance any population losses or genetic susceptibility to disease on the KP because it is nearly isolated from mainland Alaska. At least four radio-collared wolves dispersed from the KP to mainland Alaska over the past 14 years. None of several hundred wolves radio-collared outside the KP by various agencies over the same period have been reported on the KP.

As human development expands into areas adjacent to the KNWR once used by wolves and coyotes, and as human and dog use of the refuge increases, opportunities for the transmission of disease and parasites from dogs to wolves and coyotes on the refuge will continue.

Acknowledgments

We thank T.J. Roffe, J.C. Franson, and D. Doherty of the U.S. Fish and Wildlife Service, National Wildlife Health Research Center, Madison, Wisconsin for supporting and conducting the serology studies. We acknowledge the support of all KNWR managers and other staff members from 1976 to 1988 and the many KNWR and Student Conservation Association volunteers who helped capture wolves. J.D. Woolington collected serum samples between 1976 and 1980 and Alaska Department of Fish and Game personnel, especially T.H. Spraker and C.C. Schwartz, helped in many winter wolf captures. Kenai Air helicopter pilots V.L. and C.R. Lofstedt provided excellent and safe flying support during winter capture operations. Veterinarian B.L. Richards provided technical assistance and advice throughout the latter part of the study period.